Adenovirus (Fecal) ELISA Kit

Cat. No.:DEIA2435
Pkg.Size:96T

Intended use

This product is an in vitro procedure for the qualitative determination of adenovirus antigen in feces

Introduction

The adenovirus is an ubiquitous pathogen of humans and animals. Adenoviruses are characterized by location inside the cell nucleus, common complement-fixing antigens and marked stability to environmental effects. Adenoviruses are endemic in all populations throughout the year. The infection is spread both through the aerial-droplet route and the routes characteristic for intestinal infections. The incubation period is between five and seven days. Adenoviruses mainly infest respiratory and intestinal mucosa, but also the cornea. They are accumulated in the epithelial cells and regional lymph nodes. Adenoviruses cause the widest variety of illnesses of the known respiratory viruses. The adenovirus infection is the most frequently caused viral disease of the respiratory tract among preschool children (types 1 - 5 and 7). Acute diseases of the upper respiratory tract occur predominantly. Pneumonia is the most severe form of adenoviral infection occurring mostly in infants below the age of one. Adenoviruses also cause outbreaks of swimming-pool- associated pharyngoconjunctival fever in the summer and epidemics of kerato-conjunctivitis of both children and adults. The intestinal form of adenoviral infection occurs mostly in children below the age of one. An acute adenoviral infection can be detected by virus isolation and/or serology. The serologic tests are particularly important because they document actual infection in the patient and can be applied to large-scale epidemiologic investigations. The CF and ELISA tests measure predominantly the antibodies directed against the group-specific determinants on the hexon component.

Principle Of The Test

During the first incubation, adenovirus antigens present in the stool supernatant are captured by antibodies attached to the wells. The second incubation adds an additional anti-adenovirus antibody that “sandwiches” the antigen. The third incubation attaches horseradish peroxidase to the sandwich. After washings to remove unbound enzyme, a chromogen is added which develops a blue color in the presence of the enzyme complex and peroxide. The stop solution ends the reaction and turns the blue color to yellow.

Reagents And Materials Provided

1. Test Strips: Microwells containing anti-adenovirus polyclonal antibodies - 96 test wells in a test strip holder.
2. Reagent 1: One bottle containing 11 mL anti-adenovirus monoclonal antibodies with blue dye and Thimerosal.
3. Reagent 2: One bottle containing 11 mL anti-mouse antibodies conjugated to horseradish peroxidase with red dye and Thimerosal.
4. Positive Control One: vial containing 2 mL of diluted adenovirus antigen in buffer with Thimerosal.
5. Negative Control One vial containing 2 mL of buffer with Thimerosal.
6. Chromogen One bottle containing 11 mL of tetramethylbenzidine (TMB) and peroxide. Wash Concentrate (20X) Two bottles containing 25 mL of concentrated buffer and Thimerosal.
7. Stop Solution One bottle containing 11 mL of 1 M phosphoric acid.

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Materials Required But Not Supplied

1. Transfer Pipettes
2. Squeeze bottle for washing strips (narrow tip is recommended)
3. Graduated Cylinder
4. Reagent grade (DI) water

Storage

Reagents, strips and bottled components: Store between 2 – 8 ºC. Squeeze bottle containing diluted wash buffer may be stored at room Temperature.

Specimen Collection And Handling

Collection of Stool (Feces)

Stools should be collected in clean containers. Samples should be kept at 4 ºC and tested within 24 hours of collection. Samples that cannot be tested within this time should be frozen at -20 ºC until used. Freezing the specimens does not adversely affect the test. All dilutions must be made with the diluted wash buffer.

Fresh/Frozen Stools

Thaw frozen stools. Prepare a 1:5 dilution of stool by adding 1 gram (approximately the size of a pea) to 4mL of diluted wash buffer. Mix well and allow the heavy particulates to settle. For diarrheal stools a lower dilution may be used (i.e., 1:2 dilution).
Note: Do not formalize samples prior to testing.

Assay Steps

1. Break off number of wells needed (number of samples plus 2 for controls) and place in strip holder.
2. Add 100 µl of the negative control to well #1 and 100 µl of positive control to well #2 (use both as undiluted).
3. Add 100 µl of the stool supernatant to the appropriate test well.
4. Incubate at room temperature for 30 minutes, then wash. *
5. Add 2 drops of Reagent 1 (blue solution) to each well.
6. Incubate at room temperature for 5 minutes, then wash.
7. Add 2 drops of Reagent 2 (red solution) to each well.
8. Incubate at room temperature for 5 minutes, then wash.
9. Add 2 drops Chromogen to each well.
10. Incubate at room temperature for 5 minutes.
11. Add 2 drops of Stop Solution to each well. Mix wells by tapping strip holder.
12. Read results visually or on a spectrophotometer using a bichromatic reading, with the filters set at 450nm and 620-650nm. Zero the reader on air.

* Washings consist of using the diluted wash buffer to fill to the top of each well, shaking out the contents and refilling the wells for a total of 3 times.

Quality Control

The use of a positive and negative control allows easy validation of kit stability. For a valid test, the positive control must be over 0.5 OD units and the negative control must be under 0.15 OD units. Should the values fall outside these ranges, the kit should not be used.

Interpretation of Results

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Visual

Reactive: Any sample well that has distinct and substantial yellow color.

Non-reactive: Any sample well that does not have distinct yellow color.

NOTE: The negative control, as well as some samples, may show some slight color

ELISA Reader

Zero reader on air. Read all wells using a bichromatic reading with filters at 450nm and 620-650nm.

Reactive: Absorbance reading of 0.15 and above indicates the sample contains adenovirus antigen.

Non-reactive: Absorbance reading less than 0.15 indicates the sample does not contain detectable levels of adenovirus antigen.

Sensitivity

Sensitivity – 6/6 = 100%

Specificity

Specificity – 108/110 = 98%

Limitations

Test results should be used as an aid in diagnosis and should not be interpreted as diagnostic by themselves.

REFERENCES

1. Wadell, G. Laboratory Diagnosis of Infectious Diseases : Principles and Practices. New York Springer-Verlag, Volume II, 1988. Pg 284-300.
2. Hierholzer, John C. Manual of Clinical Microbiology. Washington D.C.: ASM Press, 1995. Pg. 947-955.
3. Wood, D. J. “Adenovirus gastroenteritis.” British Medical Journal, Jan. 1988; 296: 229-230.

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